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Control methods of *Botrytis cinerea*

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ABSTRACT:
Botrytis cinerea, the agent of gray mold, are attacking more of 200 plant species and produce losses in postharvest products. Methods of control of this damaging fungus are reviewed in this article. Copper derived products are used and organic fungicide of few kind (boscalid, bezimidazole, carbendazim, propiconazole, and so on) as spray, some inorganic pesticide ZnO as nanoparticles. Organic natural derived substances produced by fungi *Penicillium brasilianum Trichoderma*, and bacteria *Paenibacillus* sp., can be use against *Botrytis.* The most interesting idea is the use of antagonists (bacteria and fungi) in biological control.

Key words: grey mold, chemical methods, copper sulphate, fungicides, biological control methods.

INTRODUCTION

The gray mold is produced by *Botrytis cinerea*, attacking over 200 species of plants[1]. The authors find that an entire array of proteins are involved in virulence and heir release depends on the pH of environment which means there are a permanent adjustment of their secretome, and the production are regulated at the transcriptional level. Other consider that the number of plants species attacked is about 500 [2] and produces economic losses of about 10 to 100 billion dollars worldwide. The species is characterized by a very diverse structure, with strains dependent of host, on geography, having some genetic clusters [3]. Generally speaking, the populations of *Botrytis* a characterized by differences in morphology, reproduction, virulence [4]. They are anyway are non-homogenous species having two groups I and II, the first lacking transposable elements Boty and Flipper [5]. Analyzing the data, it is a weak association between population structure and geographical area, and a considerable difference according to the origin of the host plant [6]. Some strains looks like to be more virulent than the others, and infection incidence is dependent of temperature, at 20°C is highest, and is lower at 30°C and 5°C, the data for mycelia growth and conidial germination are similar [7]. The researches reveals the heterogeneity of field population of *B cinerea* as regarding morphology and reproduction, virulence and secondary metabolite production [4]. By molecular biological studies, some scientists revealed that *B cinerea* produced changes in proteome, the accumulation of some protein, the thickening of cell wall production of lytic enzymes, can be mechanism of host defense against attack [8. There is a different sensibility to different fungicides, depending on fungicide type and on strain of *B. cinerea*; the experiments performed in vitro showed a lower resistance to benzimidazol and carboximides and higher resistance to anilinopyrimidines [9]. Variations in population of the fungi results after point mutation changing protein structure related with the resistance [10]. The strains resistant to chemicals are the results of point

mutations and some multiple resistant strains which mechanism consists in mutations producing over expression of transporters systems members like ABC system. Another species is *B. fragariae*, attacking strawberry cultures [11] have local importance as phyto pathogen, and its presents a resistance to antifungal too. One of the elements of host (vineyard) which confers resistance to this pathogen is the wax layer of the grapes cuticula [12].

METHODS OF CONTROL OF GREY MOLD

We review the methods for *B cinerea* control , in order to compare and to discuss the necessary experiments for further development.

2.1. Copper based products

Copper based products are used for hundreds of years in Plant protection. They are based on copper sulphate, copper carbonate, copper oxychloride, copper oxychloride sulfate, copper zinc chromate, copper dihydrazine sulphate, cuprous oxide, copper hydroxide [13].

2.2. Organic pesticides

A problem of applying pesticide is the resistance to fungicides of *Botrytis cinerea*. Experiments performed in the period 2008-2011 in Italy, showed resistance of isolate in several degrees, to aniline-pyrimidines, to boscalid and others including multiple fungicide resistance, due to incorrect use of them [14]. Benzimidazole azo phenol derivatives are efficient antifungals, like compound V-5 which shown to be effective against *F. graminearum, A. solani, V. mali, B. cinerea*, and *C. lunata* [15]. Currently against *Botrytis* are used fungicides like boscalid, carbendazim, iprodone, pyrethanil and propiconazole and experiments showed the difference in their activity in inhibition of some metabolic processes [16]. ZnO, formulated as nanoparticles (NPs) can inhibits growth of Botrytis cinerea by affecting their functions and deformation of hyphae, at over 3mmol/L^{-1} to *B. cinerea* [17]. For controling of gray mold strains with multiple fungicides resistance in tomato green houses, and were used pesticides (fenxetamid and Signum- boscalid and pyraclostrobin) for control [18].

2.3. Bioactive substances

Generally we speak about natural origin compound with regulatory function, elaborated by different organisms in their antagonistic fight. *Penicillium brasilianum* produce isoroquefortine C, griseofulvin, ergosterol peroxide, 3β-hydroxy-(22E,24R)-ergosta-5,8,22-trien-7-one, cerevisterol and (22E,24R)-6β-methoxyergosta-7,22-diene-3β,5α-diol with bioactivity against fungi (*B. cinerea, Alternaria solani, Fusarium solani*, and so on) and human pathogenic bacteria [19]. Trichothecenes are produced by some fungi like *Trichoderma harzianum* CECT 2413 which intermediate compounds Trichodiene (TD) induce systemic defence [20]. Protocatehuic acid was isolated from *Paenibacillus elgii* HOA 73, and showed a potent antifungal effect against *Rhizoctonia solani* and *Botrytis cinerea* at MIC of 64 μg ml (-1) [21]. Fatty acids foods coat are used in food industry, and were tested for their action against *B. cinerea*, the results showed the reduction of its germination and attack on grapes over 54-96% compared with the control lot. In the same time FC stimulates the yeasts present on grapes [22].

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2.4. Biological controls

For biological control of this necrotrophic pathogen, were used different bacteria from *Bacillus* genus. Specialists from Algeria [23] isolated from extreme environments strains related with *B amyloliquefaciens, B. atrophaeus* and *B. mohavensis* producing proteases and cellulases, and some chitinases acting against fungi and with supplementary growth stimulating activity. The same, for the strains of *Bacillus subtilis* B27 and B 29 which reduced attack to grapevine up to 50 -60%, due to chitinolytic enzymes [24]. *Bacillus amyloliquefaciens* subsp. *plantarum* strains IMV B-7404 and BIM B-439D demonstrated an antagonism against vascular bacteriosis agent and produced inhibition of *B cinerea* and *Pencillium expansum* [25]. The *Bacillus* strains are able to produced lipopeptides which can be a tool for biocontrol of postharvest phytopathogens [26]. Yeasts isolated from grapes and wine, can be use as antagonists of *B. cinerea* and from abnout 591 isolates *Aureobasidium pullulans, Metschnikowia pulcherrima* and *Pichia guilliermondii* showed a good antagonistic effect even better thet the *Candida oleophila* which is used usually for biocontrol [27]. Yeast *Candida sake* was effective against grey mould of tomato [18]. Other yeast strains with bio-control capacities are *Saccharomyces cerevisiae, Wickerhamomyces anomalus, Metschnikowia pulcherrima Aureobasidium pullulans,* strains of the first and the second, having the best antifungal activity against Botrytis cinerea [28].*Trichoderma* spp. [29] has potential as biocontrol agent for *Botrytis cinerea* .

Some bacteria can protect plants from *B cinerea* attack which synthesize anti-fungal metabolites, induce host resistance and are competitive for nutrients with fungi [30]. A strain of *Brevibacillus brevis*, W4 [31], has a clear inhibitory effect on *B. cinerea.*

Actinomycetes are known as releasing precious secondary metabolites. *Streptomycin patents* LMM15 (its fermented substrate) can inhibits growth of grey mould and reduce lesions formed on tomato leaves and fruits by increasing content of proline and malon di-aldehyde [32], providing the defense of plants.

Some fungi can defend the plants against grey mold too. *Trichoderma* sp and *Clonostachys* sp strains reduced mortality of mini cutting of *Eucalyptus globulus* in forest nurseries reducing disease in the same results like commercial fungicides [33]. Following experiments performed in vitro and in vivo, *Burkholderia cepacia* Cs5 demonstrated antagonistic capacity on fungus *B. cinerea* [34], being a possible future bio-fungicides.

CONCLUSIONS

The fight against this necrotrophic fungi is a real challenge. The chemical methods of control are generally applied but it's affecting the environment despite all preventive measure taken and possible the consumer's safety. Practically a better method is the biological control, using antagonistic microorganisms. The examples e described in the present work, prove that this is really possible, being cheap, safe for man and environment and effective against *Botrytis cinerea* and possible other phytopathogenic fungi. There are still many aspects to be clarified by further researches.

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